Journal of Radioanalytical and Nuclear Chemistry, Vol. 269, No.2 (2006) 267–270

Stable isotope tracer marking of individual boll weevils

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(Received April 6, 2006)

Stable isotope markers have been used to study animal nutrition for several decades and more recently to study the foraging and cultural habits of imported fire ants. In this work, we have extended that effort to evaluate the potential for marking boll weevils, *Anthonomus grandis grandis Boheman (Coleoptera: Curculionidae)*, with the rare earth element samarium to aid in studies of insect invasion and pest eradication protocols. Neutron activation analysis (NAA) was performed on the marked boll weevils as well as plant material from the cotton squares on which the insects were fed. Samarium levels in non-dosed insects average about 20 ng/g or about 100 pg total element per insect. Our computed average determination limit was 36 pg samarium/weevil. The determination limit for cotton plant squares and leaves averaged 3.5 ng/g and 8.2 ng/g, respectively. These initial results indicate the NAA method is capable of identifying individual marked insects which have assimilated 1 ng of samarium, a ten-fold increase in content over average blank values.

Introduction

The Mexican boll weevil, *Anthonomus grandis grandis Boheman (Coleoptera: Curculionidae),* began its infestation of the southern United States in the 1870s and was first identified in Texas in 1892.[1] The cotton industry was quickly crippled nationwide with typical crop losses of seven to ten percent and $200 million per year by the 1930s. Eradication efforts, begun in the 1960s have effectively controlled the insects in many of the southeastern states but are incomplete in Texas.[2] Eradication phases, including maintenance in those states which have been designated as “suppressed” or “complete”, require active control and monitoring efforts.[3] The ability to trace individual insects would add an important tool for studying the behavior and migration of boll weevils.

Our laboratory has used rare earth stable isotope markers for many years to study animal nutrition, primarily in cattle.[4–6] We have also used such markers more recently to study the foraging behavior and cultural habits of the imported fire ant.[7,8] In those studies we tracked markers ingested by fire ants by trapping and conducting elemental analysis using neutron activation. The boll weevil provides the possibility of expanding this technique to another important imported pest. Since the boll weevil is considerably larger than the ants previously studied (weevil body masses averaged 3.9 mg), the potential of tracking individual insects is enhanced.

In the present study, experiments were performed to determine optimal dosage in artificial boll weevil diet and duration of exposure for the element to subsequently be detected and quantified. In addition, experiments were conducted to measure persistence of the element in the insect body. Finally, effectiveness of marking the weevils by allowing them to feed on cotton plants grown under laboratory conditions and irrigated with samarium-containing water was studied.

Experimental

Sampled materials

Groups of five laboratory-reared boll weevils were fed with artificial boll weevil diet that included samarium at three concentrations and for two durations. Boll weevil diets labeled at dosages of 0.01%, 0.1% and 1.0% by weight were fed to groups of insects for a period of either 2 or 7 days. One group from each diet was then collected immediately to evaluate the effectiveness of dosage levels. Other groups were collected 5 or 10 days after being removed from the marked diet and provided non-marked food to study persistence of samarium in the body of the insect. Additional experiments were conducted in which the weevils were fed on cotton squares from plants irrigated with three concentrations of samarium-dosed water, in order to evaluate the effectiveness of marking insects by ingestion of cotton plant material. Groups of weevils were fed from plants irrigated with samarium at 0.01%, 0.1% and 1.0% aqueous samarium solutions. Boll weevils were then collected as well as cotton plant components to evaluate uptake of samarium by the plant and its availability to the insect. Table 1 summarizes the samples analyzed in the insect experiments.

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0236–5731/USD 20.00

Akadémiai Kiadó, Budapest
Springer, Dordrecht

Table 1. Samples collected for study. Plant material was analyzed as dry matter; insects were analyzed as collected

Dosage and persistence study				
Duration of diet, days	Sm* concentration of diet, %	Delay before collection, days	Sample matrix	Replicates, No.
0 (control)	0 (control)	n/a	Boll Weevil	5
2	0.01	0	Boll Weevil	5
2	0.01	5	Boll Weevil	5
2	0.01	10	Boll Weevil	5
2	0.10	0	Boll Weevil	5
2	0.10	5	Boll Weevil	5
2	0.10	10	Boll Weevil	5
2	1.0	0	Boll Weevil	5
2	1.0	5	Boll Weevil	5
2	1.0	10	Boll Weevil	5
7	0.01	0	Boll Weevil	5
7	0.01	5	Boll Weevil	5
7	0.01	10	Boll Weevil	5
7	0.10	0	Boll Weevil	5
7	0.10	5	Boll Weevil	5
7	0.10	10	Boll Weevil	5
7	1.0	0	Boll Weevil	5
7	1.0	5	Boll Weevil	5
7	1.0	10	Boll Weevil	5
Delivery study				
Duration feeding on plant, days	Sm concentration of irrigation, %	Delay before collection, days	Sample matrix	Replicates, No.
0 (control)	0 (control)	0	Boll Weevil	10
2	0.01	0	Boll Weevil	10
2	0.10	0	Boll Weevil	10
2	1.0	0	Boll Weevil	10
Plant uptake study				
Sm concentration of irrigation, %	Sample matrix	Replicates, No.	Sample matrix	Replicates, No.
0 (control)	Square	5	Leaf	5
0.01	Square	5	Leaf	5
0.10	Square	5	Leaf	5
1.0	Square	5	Leaf	5
Total No. of samples				175

* Sm: Samarium.

Neutron activation analysis

Samarium was chosen for use in these experiments due to its extremely high sensitivity for detection with neutron activation analysis (NAA). The neutron activation protocol developed followed that used earlier for the fire ant studies published elsewhere.[7,8] In brief, samples were prepared by encapsulation in pre-cleaned polyethylene irradiation vials. Comparator standards were prepared by evaporation of weighed quantities of primary samarium standard solutions onto cellulose. Standards were prepared at levels ranging from 1 to 100 ng samarium. National Institute for Standards and Technology (NIST) Standard Reference Material SRM 1571, orchard leaves, with a known samarium content of 114±20 ng/g,[9] was included with each batch of eight weevil or cotton plant samples to provide quality assurance of accuracy. Samples, standards and quality control (QC) materials were then irradiated together in the Texas A&M Nuclear Science Center's 1 MW research reactor facility for 14 hours at a nominal neutron flux of $1 \cdot 10^{13}$ n·cm^{-2}·s^{-1} in a rotisserie position. All irradiated materials underwent gamma spectroscopy after decay periods of 2 to 6 days, depending on the sample matrix. Spectra were accumulated for 1 hour on a Canberra Industries Alpha-based VMS Genie system using high purity germanium detectors from Canberra or Ortec/Ametek. The 103 keV gamma-line from ^{153}Sm

was utilized for comparison of samples to standards and computation of samarium content was accomplished using Canberra's Genie NAA software.

Results and discussion

Development of the method

Limits of detection and quantification were measured for boll weevils ($n = 6$), cotton plant squares ($n = 7$) and plant leaves ($n = 6$). The determination limit in boll weevils averaged 36 pg. The activity generated in the matrix in the case of the plant material, primarily sodium, resulted in the higher limits of 122 and 117 pg for squares and leaves, respectively. These values correspond to concentrations of 3.5 and 8.2 ng/g dry weight, respectively, for the sample masses used in this study.

Fifteen boll weevils were analyzed for samarium as control insects. The samarium content determined for these controls ranged from undetectable quantities (two cases) to 220 pg. Due to the variability of samarium in the control animals, it is suggested that the minimum assimilation of marker for identification of test insects should approach 1 ng, a ten-fold increase over our average blank value.

Samarium concentration in the QC material, NIST SRM Orchard Leaves was measured a total of 21 times. As shown on the control chart in Fig. 1, the mean result was 111.5±6.3 ng/g which deviated from the literature value of 114 ng/g by about 2.8%.

Test materials

The focus of the present communication is related to development of the analytical method. Therefore, a detailed analysis of the biological impact is not provided here. However, Table 2 shows typical values obtained. The level of dosing was sufficient for the insects to assimilate detectable quantities of samarium. In all experiments in which insects were fed on samarium-laced diets, samarium was detected in quantities far exceeding the methods determination limit. However, marker concentrations dropped rapidly after the marked diet was removed from the weevils. Table 2 also indicates that the method satisfactorily measured the samarium marker in the cotton plants grown with laced irrigation, however, little of that marker seemed to be assimilated by the insects feeding on the cotton.

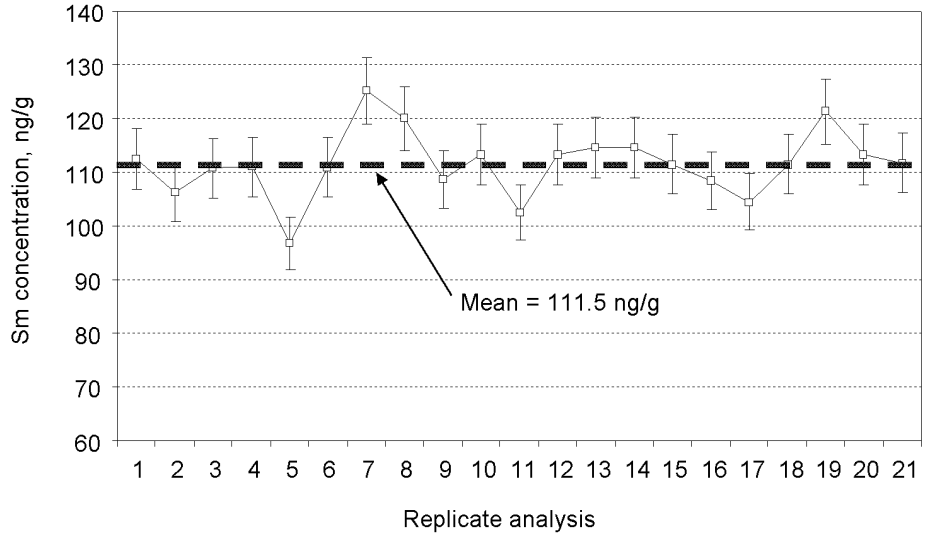

Fig. 1. Control chart for quality control material, National Institute for Standardization and Technology, Standard Reference Material 1571, Orchard Leaves. The mean result of 21 replicate determinations of 111.5±6.3 ng/g differs from the literature value of 114±20 ng/g by 2.8%

Table 2. Samarium measurement mean ranges for weevils collected immediately following removal from marker and for cotton plant components. Range generally correlates with dose of samarium administered

Sample matrix	Samarium range
Weevils fed Sm-laced diet	13–287 ng
Weevils fed from Sm-irrigated cotton	95–205 ng
Cotton squares	4–422 ng/g
Cotton leaves	12–3950 ng/g

Conclusions

The use of samarium as a stable isotope marker has once again proven to be advantageous due to the extreme sensitivity of determination of the element with instrumental NAA. Sensitivity of the method is sufficient to measure as little as 1 ng of marker in the body of a single individual boll weevil insect. This corresponds to one part in about 4000 total body mass for our test insects with an average weight of 3.9 mg. The application of this methodology to marking boll weevils suffers, however, due to the extremely small assimilation and rapid clearing of the element in the test animals.

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The authors wish to acknowledge support from the Office of the Vice President for Research at Texas A&M University for many of the neutron irradiations and the Nuclear Science Center for providing those services.

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